

**United States Department of Agriculture
Agricultural Marketing Service, Science & Technology
Microbiological Data Program**

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Title: Procedure for Testing and Maintaining Control Strains		
Revision: 04	Replaces: Rv 03 05/01/06	Effective: 04/01/2009

1. Purpose

To provide standard procedures for testing and maintaining all control strains.

2. Scope

This Standard Operating Procedure (SOP) shall be followed by all laboratories conducting microbiological studies for the USDA/AMS Microbiological Data Program (MDP), including support laboratories conducting non-routine activities that may impact the program. This SOP represents minimum MDP requirements and is presented as a general guideline. Each laboratory shall have written procedures that provide specific details concerning how the procedure has been implemented in that laboratory.

All MDP laboratories shall use the control strains specified in SOP MDP-QA-03. Strains are preserved as **master stock cultures** for maintenance and **working stock cultures** for testing and distribution. If problems are encountered with any control strain, MPO shall be notified. The MDP (Ohio) Culture Repository will ship a new stock to replace the problem stock.

3. Outline of Procedures

Equipment and Materials	5.1
Revival and Growth of Control Strains (by MDP Culture Repository only)	5.2
Maintenance, Storage and Testing of Cultures (by MDP (Ohio) Culture Repository and MDP participating laboratories)	5.3
Revival and Growth of Control Strains (by MDP participating laboratories)	5.4

4. References

- 4.1 Bacteriological Analytical Manual (BAM) Online, 8th edition. 2001. US FDA/CFSAN. <http://www.cfsan.fda.gov/~ebam/bam-toc.html> (last accessed 3/09)
 - 4.1. Monday, SR, Weagant, SD and Feng, P. 2003. Use of endogenous host plasmids for generation of *Escherichia coli* O157:H7 and *Shigella sonnei* that stably express the green fluorescent protein. *Plasmid*. 50: 161-167.
 - 4.2. Rang, C, et.al. 2003. Fitness cost of the green fluorescent protein in gastrointestinal bacteria. *Can. J. Microbiol.* 49: 531-537.
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4.3. SOP MDP-QA-03, Quality Assurance (QA) Controls

4.4. Microbank™ Microorganism Storage System user Instructions

5. Specific Procedures

5.1. Equipment and Materials

5.1.1. Positive and negative control bacterial strains for each test method, as detailed in SOP MDP-QA-03

5.1.2. Materials, media and reagents required for testing routine MDP produce samples

5.1.2.1. For cultural media and growth conditions pertaining to individual strains, refer to method SOPs listed in the Reference section

5.1.2.2. Incubator set at $35 \pm 2^{\circ}\text{C}$. Refer to individual method SOPs as listed in the Reference section.

5.1.2.3. Long wavelength UV light source

5.1.2.4. Microbank storage beads, Pro-Lab Diagnostics, <http://www/pro-lab.com>,

5.1.2.5. Kanamycin, Sigma Catalog # K1377 and Isopropyl- β -D-thiogalactopyranoside (IPTG), Sigma Catalog # I5502.

5.1.2.5.1. Kanamycin: Prepare stock solution, filter sterilize using a 0.2 μm syringe filter (B-D # 309602 - Fisher Catalog # 09-719C or equivalent), and store at $2-8^{\circ}\text{C}$ (for 3 weeks maximum) or -20°C or lower for long-term storage. Add appropriate amount to sterile culture medium (after cooling to $\sim 50^{\circ}\text{C}$) for a 50 $\mu\text{g}/\text{mL}$ final concentration.

5.1.2.5.2. Isopropyl- β -D-thiogalactopyranoside (IPTG): Add 0.24 gram of IPTG to sterile culture medium (after cooling to $\sim 50^{\circ}\text{C}$) for a 1 mM final concentration.

5.1.2.6. Nutrient agar plates containing 50 $\mu\text{g}/\text{mL}$ kanamycin

5.2. Revival and Growth of Control Strains (by MDP Culture Repository only)

5.2.1. For strains sent on discs (e.g., 1 cm diameter discs): Place the disc on a Nutrient agar plate or suitable rich media. Wet the disc with a small volume (0.1 mL) of sterile solution such as saline, Phosphate Buffered



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Saline, or any rich broth using a sterile pipette or a micropipette with a sterile tip. Using a sterile loop, streak the solution from the disc across the agar surface for colony isolation. Invert the plate and incubate for at $35 \pm 2^{\circ}\text{C}$ 18-24 hours.

- 5.2.2. For strains sent in stab-vials: Using a sterile loop, remove cells from the stab and streak on a Nutrient agar plate for colony isolation. Invert the plate and incubate at $35 \pm 2^{\circ}\text{C}$ for 18-24 h.
- 5.2.3. For strains sent on slants: Using a sterile loop, streak on a Nutrient agar plate for colony isolation. Invert the plate and incubate at $35 \pm 2^{\circ}\text{C}$ for 18-24 h.
- 5.2.4. For strains sent on Microbank™ beads: Aseptically remove one bead. Immediately close the vial tightly and return it to low temperature storage as soon as possible. Streak on a Nutrient agar plate or drop into a rich broth and incubate at $35 \pm 2^{\circ}\text{C}$ for 18-24 h.
- 5.3. Maintenance, Storage and Testing of Cultures (by MDP Culture Repository and MDP participating laboratories)
 - 5.3.1. Upon receipt, control strains that carry the kanamycin resistance (kan^{R}) gene should be grown on a non-specific rich media containing kanamycin to ensure that the plasmid is maintained or the genes are integrated into the chromosome. Strains that do not carry genes coding for kan^{R} can be grown in the non-specific rich medium without kanamycin and inoculated into the specific pre-enrichment media.
 - 5.3.2. Storage of the cultures: For making master and working stocks of strains, follow Microbank™ beads manufacturer's directions (refer to product insert for additional details):
 - 5.3.2.1. Label appropriate Microbank™ vials with the name of organism, date, vial number (i.e. 1 of 5) and initials.
 - 5.3.2.2. To prepare stocks, follow manufacturer's instructions to inoculate Microbank™ vials. Inoculate desired number of cryovials (minimum requirement: 1 vial for reference and 2 vials as working stocks).
 - 5.3.2.3. Store at -70°C or lower.
 - 5.3.3. Tests

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Test control strains when they are received from the Culture Repository or other sources, and when stocks for long term storage are prepared

5.3.3.1. For detection of control strains that carry genes coding for GFP in broth or on agar, the cultures can be exposed to long wavelength UV at 365 nm. These cultures will show green to bluish-green fluorescence, indicating the expression of the gene coding for GFP. Occasionally, the intensity can be enhanced by holding the cultures at room temperature for 1-2 hours following routine incubation followed by examination with UV light. Cultures grown on blood agar plates may fluoresce better than cultures grown on selective agar plates or Nutrient agar plates. For control strains (e.g. MDP-004, *E. coli* O157:H7 carrying the gene coding for GFP) in which the expression of the gene coding for GFP is under the control of *lac* or *tac* promoter, addition of 1 mM IPTG to cultures will enhance the fluorescence.

5.3.3.2. For additional strain characteristics, refer to SOP MDP-QA-03.

5.3.3.3. Laboratories should verify the purity of control strains prior to use and periodically according to internal laboratory procedures.

5.4. Revival and Growth of Strains (by MDP participating laboratories)

5.4.1. Control strains are revived from Microbank™ long-term storage on a monthly basis during routine testing.

5.4.1.1 Aseptically remove one Microbank™ bead and transfer to Nutrient media with or without kanamycin (refer to 5.3.1). Non-specific rich medium can be used for subsequent transfers.

5.4.2. To minimize contamination risk and genetic drift, cultures should not be subjected to more than four passages after their revival from long term storage.

5.4.3. Each laboratory shall have internal written procedures for storage, handling and maintenance of QC cultures.



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4/1/09

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Revision 04 March 2009 Monitoring Program Office

- Added 4.4 and 4.5 to the Reference section.
- Deleted archived SOPs and non-referenced SOPs from the Reference section.
- Added 5.1.2.4 to section 5.1
- Added, “such as” to 5.2.1.
- Changed “scoop out” to “remove” in 5.2.2.
- Changed “scrape cells using” to “Using” in 5.2.3.
- Added, “Upon receipt” to 5.3.1.
- Changed “cryo” to “appropriate” in 5.3.2.1 and changed “passage number” to “vial number (i.e. 1 of 5)” to 5.3.2.1.
- Deleted “cryo” from 5.3.2.2.
- Changed, “other” to “additional” in 5.3.3.2.
- Deleted 5.3.3.3 and added, “Laboratories should verify the purity of control strains prior to use and periodically according to internal laboratory procedures”.
- Deleted “This can be achieved by removing a bead from the Microbank vials, or inoculating from cryovials with preserved cultures under aseptic conditions” from 5.4.1.
- Revised 5.4.1.1 to read, “Aseptically remove on Microbank bead and transfer”.
- Added 5.4.3.

Revision 03 March 2006 Monitoring Program Office

- Updated References (4.0), Equipment and Materials (5.1)
- Revised instructions for the preparations of kanamycin and Isopropyl-β-D-thiogalactopyranoside (IPTG) to reflect current practices
- Deleted Control Maintenance for MDP Control Strains table
- Revised Maintenance and Testing of Cultures (5.3) and Revival and Growth of Strains (5.4) sections

Revision 02 January 2005 Monitoring Programs Office

- Updated specific strains
- Changed concentration of kanamycin as antibiotic supplement

Revision 01 July 2004 Monitoring Programs Office

- Eliminated tetracycline stock preparation instructions
- Deleted unused control strains, MDP-006 and MDP-007

